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Vitamin D enhanced pork from pigs exposed to artificial UVB light in indoor facilities

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Abstract

Vitamin D deficiency is a recognized problem in Europe; this can be minimized by fortifying a broader range of foods. Our aim was to investigate the potential for enhancing the vitamin D content of pork from pigs raised in indoor facilities, by exposing the pigs to UVB for a period just before slaughter. Three groups of six pigs were exposed to 0, 0.7 or 1 SED/day for 28 days. A fourth group was exposed to 2 SED; this treatment was not completed due to mild erythema. The highest increase of vitamin D_3 was achieved with 1 SED; the vitamin D_3 content in loin was 3.7 ng/g; more than a factor of 2 higher compared to previously reported results from studies using 2000 IU/kg feed, the maximum allowed level in Europe. This is the first time an increase in the vitamin D content of pork has been reported as a result of using artificial UVB-exposure of slaughter pigs in indoor facilities. However, the maximum production of vitamin D was probably not reached as a linear relationship between UVB dose and vitamin D content was found; therefore, the UVB-lighting method described still calls for further investigation to realise its full potential to enhance vitamin D in pork.

Keywords

Vitamin D; UVB; Pork; Pigs

Introduction

On annual basis around 13 % of the European population is experiencing vitamin D deficiency (vitamin D status < 30 nmol/L) and 40 % vitamin D insufficiency (vitamin D status < 50 nmol/L) [1]. Although the recommended intake of vitamin D is between 10-20 μg/day [2, 3], the estimated intake is only around 3-7 μg/day[4]. It has been shown that vitamin D intake may be increased by effective food-based strategies [5, 6]. A feasible strategy is to fortify a broader range of foods and/or increase the natural content of vitamin D in foods, e.g. fatty fish, eggs and meat [4, 7, 8].

Adding vitamin D to the final food product e.g. milk dairy products, and breakfast cereals is the traditional way of fortifying [9]. An alternative is using the natural mechanisms to enhance the vitamin D content in foodstuff;. Like humans, livestock are able to produce vitamin D_3 in the skin when exposed to UVB light (290-320 nm) either artificially or from the sun [10–15]. Also when adding more vitamin D to the livestock feed the vitamin D content in the final food product will increase [16, 17].

Vitamin D exists in two forms, vitamin D_2 and vitamin D_3. Vitamin D and 25-hydroxyvitamin D (25(OH)D), the major metabolite of vitamin D, is naturally found in products of animal origin; although there are other metabolites only 25(OH)D is so far included when determining the total vitamin D content in food [18, 19].

EU has maximum limits for adding vitamin D to feed. For pigs this limit is 2000 IU/kg feed (Commission Directive 91/248/EEC), that is reported to produce lean pork loin with a content of 1.2 ng vitamin D_3/g and liver with a content of 8.4 ng vitamin D_3/g [17]. In Denmark, the pigs are not fed with the maximum allowed level of vitamin D as the recommended level for pigs (30-100 kg) is 400 IU/kg feed [20], that result in lean pork with a vitamin D_3 content of 0.24 ng/g [21]. This is in contrast to earlier findings from our research group where a vitamin D_3 content of 5-11 ng/g and 42 ng/g was measured in muscle tissue and in liver, respectively, from mini-pigs after they had been exposed to UVB light; the daily exposure was similar to 10-20 min summer sun at 55 °N, for 120 days [10].

Already in 1924 it was shown that pigs produce vitamin D when exposed to sunlight [22]; and in 1925 it was reported that indoor livestock production reduces the vitamin D content in animal products for human consumption [23]. A recent study shows that the vitamin D content will increase in the meat when pigs are exposed to sunlight during the summer; specifically1 hour of summer sunlight per day for14 days before slaughter resulted in lean pork loin with a content of 7.2 ng vitamin D_3/g [13]. However during winter if above or below 35 °N and 35 °S, respectively, no cutaneous vitamin D will be produced [24]. Giving pigs' access to an outdoor area will therefore not alleviate the problem with human vitamin D deficiency, which is a problem, especially in winter.

The aim of this study was to investigate the potential to enhance the vitamin D content of pork from pigs raised in indoor facilities, by exposing the pigs to UVB-light for a period just before slaughter.

Materials and methods

Animals and test environment

The study included 24 slaughter pigs, all females and fair-skinned, of the Danish breed LYD (Landrace, Yorkshire and Duroc); purchased from Askelygård, Roskilde, Denmark. At delivery the pigs had a mean weight of 50 kg (range 44-79 kg). All pigs were clinically healthy at arrival.

The pigs were allocated at random into 4 groups of 6 and placed in pens of 3x4.5 m, which were separated by UV-impenetrable walls.

The pigs were fed standard feed with a vitamin D_3 content of 420 IU/kg (Danish Top S1 611+3+5, Danish Agro, Karise, DK). The feed was administered twice a day. In the last 28 days of the trial the amount was up to 1 kg per pen per feeding. Water was offered ad libitum, and fresh straw was provided every day. The stable was lit by fluorescent tubes from 8:00 to 14:30 during the entire study. The fluorescent tubes did not emit any UVB light.

The trial ran from November 2016 to February 2017. The pigs were kept at the research facility for large animals at University of Copenhagen (Rørrendegård, Taastrup, Denmark).

Study design and sample size determination

The UVB-treatment was a parallel study with three treatment groups and one control group; it lasted 28 days. Based on sample size calculation with α=5 %, 1-β=0.8, SD=24 % [16] and an expected increase of 50 %, a group size of four was needed; however six were chosen to be on the safe side.

UVB-exposure

In each of the pens housing a treatment group, four UVB tubes (Lucky Reptile UV sun T5, 54 W tubes, luckyreptile.com, Germany) were placed at a height of approximately 180 cm above the floor. Normal stable armatures were used, but without plastic covers, as they absorbed the UVB-light. The pigs in the 3 treatment groups were gradually adapted to UVB light over the first 8 days of exposure. The doses are stated in units of standard erythemal dose (SED) where 1 SED is equivalent to 100 J/m^2 erythemal dose [25]. During the first 7 days the 3 treatment groups were given the same doses: day 1-2, 0.3 SED; day 3-5, 0.6 SED; day 6-7, 0.7 SED. From day 9 the remaining 2 groups were exposed to the planned doses of 1.0 SED and 2.0 SED, respectively, while the third group continued with 0.7 SED. The control group received no UVB exposure. The pigs were slaughtered on day 29, after 28 days of UVB-exposure.

UVB measurements

At DTU Fotonik, Department of Photonics Engineering, Technical University of Denmark, a full spectrum of the irradiance was recorded for one tube, scanning from 200 nm to 700 nm, with 1 nm increment (EOP146 detector probe, Instrument Systems-CAS140CT). The tube emitted UVB from 286 nm to 320 nm, and had a maximum at 312 nm. The full spectrum is shown in Online Resource 1.

The erythemal effective irradiance was calculated using the measured spectrum and the erythema reference action spectrum [25] which couples irradiance to the development of erythema. A figure of the measured spectrum with the erythema reference action spectrum superimposed is shown in Online Resource 2. For measurements in the stable a handheld ILT 1400-BL photometer equipped with a SEL005/TLS312/TD detector (International Light Technologies, Peabody, MA) was used to measure the irradiance at 16 positions at two distances from the floor; 30 cm and 60 cm, to illustrate pigs lying and standing. The average of the 32 measurements combined with the calculated erythemal effective irradiance was used to assess the exposure times of 150, 214, and 427 minutes, in order to achieve the target doses of 0.7, 1 and 2 SED, respectively.

Weighing

The pigs were weighted 7 days before the UVB treatment started and just before slaughter.

Slaughtering process

The 24 pigs were electrically stunned, then stunned with a captive-bolt pistol, pithed and then hoisted by one leg and stuck. After bleeding, the pigs were scalded and dehaired in a scalding tub, followed by singeing using a hand-held gas torch. After singeing, black deposits and singed hairs were scraped off with a knife and washed off before evisceration; the liver was collected and kept at -20 °C until further treatment. The carcasses were cut in half and hung in cold storage overnight.

Sampling and homogenization

The day after slaughter, half of each carcass was divided into primal cuts and kept at -20 °C until further treatment within four days. The middle third of the pork loin with rind (Longissimus dorsi with subcutaneous fat and skin; approximately 1.2 kg) was carefully divided into lean meat, subcutaneous fat and rind. Before analysis the liver, lean meat and subcutaneous fat was homogenized (Tecator 1094 Homogenizer (Foss Tecator, Höganäs, Sweden) and Bamix® SliceSy®, respectively). The rind was divided into 20 strata, and from each stratum a random small biopsy (diameter: 4 mm) was taken and combined into one sample.

Vitamin D analysis

The analytical method used on tissue samples, which included vitamin D_3, 25(OH)D_3, vitamin D_2 and 25(OH)D_2, was developed by combining and optimizing different parts of two published methods for the quantification of vitamin D_3 and 25(OH)D_3 in serum and tissue [26, 27]. A full description of the method is available in Online Resource 3. In short, the food samples were saponified over night at room temperature, clean-up by liquid-liquid extraction and solid phase extraction. For quantification on LC-MS/MS vitamin D metabolites were derivatized by 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). In serum samples the protein was precipitated and clean-up by solid phase extraction.

The precision of the method was < 10 % in food and < 5 % in serum. Furthermore the correctness of the method was checked by analyses of certified reference materials (Milk powder, CRM421, IRMM, Geel, Belgium) and recovery of added vitamin D. The result was 151 ng vitamin D_3/g ± 2.4 % (n=8) which was within the certified values of 0.143 ±

0.008 mg/kg, while the recovery for vitamin D_3, vitamin D_2, 25(OH)D_3 and 25(OH)D_2 was 90-127 % (n=9). All of the analyses were conducted in laboratory environment accredited to perform the analyses according to ISO17025 [28].

Content of fat

The fat content was determined gravimetrically using a Schmid-Bondynski-Ratslaff (SBR) method [29]. The sample was boiled in hydrochloric acid, ethanol was then added and the lipids were extracted using diethyl ether:petroleum ether (1:1) that was subsequently evaporated and the fat was weighed.

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey tests was used to determine significant difference in the vitamin D content between the individual groups using 95 % family-wise confidence interval. The means were log transformed to fulfill the assumption of equal variance and normal distribution; except for vitamin D_2 in liver. The 2 SED group was excluded from the analyses as the treatment was not completed. One-way ANOVA was used to test for difference in fat content between the groups. The statistical analyses were performed in R [30] using RStudio [31]. Association between vitamin D status and content of vitamin D in other parts of the pigs were assessed with Pearson's correlation coefficient using the Data Analysis function in Excel (version 2010, Microsoft, Redmond, WA). Data is reported as mean ± standard deviation (SD).

Results

Clinical findings

No effect on health or behavior was observed in three of the groups: control, 0.7 SED and 1 SED. The pigs in the 2 SED group developed mild erythema on the ears and backs after 10 days with the dose of 2 SED, i.e. day 19, therefore, the UVB treatment was immediately ceased for this group. The veterinarian did not find the redness severe and the general well-being and behavior of the pigs were normal; the pigs were therefore kept alive until the end of the trial and

slaughtered together with the other pigs at day 29. Thus this group did not complete the treatment and was left out from further analyses.

Content of vitamin D and fat

The measured content of vitamin D_3, $25(OH)D_3$, vitamin D_2, $25(OH)D_2$ is displayed in Table 1 from the three groups that completed the treatment. The main finding was that the treatment with 1 SED resulted in the highest content of vitamin D_3; specifically the content in rind, subcutaneous fat, lean meat, and liver was 316 ng/g, 120 ng/g, 3.7 ng/g, and 29 ng/g, respectively.

Table 1. Content of the four vitamers in rind, subcutaneous fat and lean meat from pork loin, in liver and in serum, and fat percentage for the samples from loin from pigs treated with different doses of UVB for 28 days.

	0 SED		0.7 SED		1 SED	
	Mean	SD	Mean	SD	Mean	SD
Rind						
Fat (%)	11.7[a]	2.3	11.0[a]	2.4	12.2[a]	2.8
Vitamin D_3 (ng/g)	1.7[a]	0.2	146[b]	76	316[c]	159
$25(OH)D_3$ (ng/g)	2.0[a]	1.0	12[b]	4	21[c]	8
Vitamin D_2 (ng/g)	0.5[a]	0.1	0.8[b]	0.2	1.1[b]	0.3
$25(OH)D_2$ (ng/g)	0.9	0.3	< 0.5		< 0.5	
Subcutaneous fat						
Fat (%)	77.9[a]	5.5	74.5[a]	5.1	78.4[a]	4.7
Vitamin D_3 (ng/g)	4.1[a]	1.1	66[b]	21	120[c]	56
$25(OH)D_3$ (ng/g)	0.9[a]	0.2	4.1[b]	0.8	6.9[c]	1.8
Vitamin D_2 (ng/g)	1.7[a]	0.9	2.6[b]	1.0	2.8[b]	0.7
$25(OH)D_2$ (ng/g)	< 0.5		< 0.5		< 0.5	
Lean meat						
Fat (%)	1.9[a]	0.6	1.8[a]	0.3	1.5[a]	0.2
Vitamin D_3 (ng/g)	0.2[a]	0.03	2.3[b]	0.4	3.7[c]	1.0
$25(OH)D_3$ (ng/g)	0.3[a]	0.08	1.6[b]	0.2	2.4[c]	0.8
Vitamin D_2 (ng/g)	0.3[a]	0.08	0.4[b]	0.1	0.5[b]	0.1
$25(OH)D_2$ (ng/g)	< 0.5		< 0.5		< 0.5	
Liver						
Vitamin D_3 (ng/g)	0.8[a]	0.3	14.9[b]	3.8	29[c]	9
$25(OH)D_3$ (ng/g)	0.8[a]	0.2	4.9[b]	0.8	8.3[c]	2.4
Vitamin D_2 (ng/g)	0.7[a]	0.6	2.3[b]	0.3	2.7[b]	0.4
$25(OH)D_2$ (ng/g)	< 1.0		< 1.0		< 1.0	
Serum						
Vitamin D_3 (ng/ml)	0.8[a]	0.3	17[b]	4	31[c]	9

$25(OH)D_3$ (ng/ml)	11^a	3	52^b	8	95^c	25
Vitamin D_2 (ng/ml)	0.4^a	0.2	0.8^b	0.1	1.0^b	0.3
$25(OH)D_2$ (ng/ml)	5.1^a	1.3	2.3^b	0.2	2.2^b	0.4

[a,b,c] Mean values within a row with unlike superscript were significantly different (One-way ANOVA with Tukey test, $p < 0.05$).

There was a significant association between the serum $25(OH)D_3$ (vitamin D status) and the vitamin D_3 content in the other tissues of the pig (Pearson's R 0.92-0.98). A table with the exact values is available in Online Resource 4.

There was also a significant association between the UVB dose and the content of vitamin D_3 and $25(OH)D_3$ in all parts of the pig (Pearson's R 0.96-1.00). The vitamin content as a function of UVB dose is shown in Online Resource 5.

Weight

The average live weight at slaughter was 112±6 kg; equal to the average live weight of slaughter pigs in Denmark. The average weight gain during the UVB treatment was 22±3 kg; there were no significant difference between groups.

Discussion

The main goal of this study was to investigate the potential to enhance the vitamin D content of pork from pigs raised in indoor facilities, by exposing pigs to artificial UVB for a period just before slaughter.

This is the first study that reports data on the effect of different UVB-exposure levels in slaughter pigs. The doses of 0.7 SED, 1 SED and 2 SED were chosen to simulate approximately 7 to 20 minutes of midday sun exposure during summertime in Denmark (56 °N) [32]. We had planned to investigate the effect of increasing the dose to a level of 2 SED, but unfortunately the treatment with 2 SED had to be stopped, due to mild erythema (see Clinical findings in the Results section). However, the results for 0, 0.7 and 1 SED showed that the vitamin D_3 and 25-hydroxyvitamin D_3 content in the analysed parts of the pig was linearly associated with the daily exposure time with a Pearson coefficient > 0.96. This indicates that the maximum production of vitamin D has not been reached using 1 SED as the vitamin D production after exposure to UVB has been shown to be non-linear in humans and hens [33, 34].

In an earlier study from our research group, mini-pigs did not develop erythema when exposed to a UVB dose of 0.9 SED for 84 days followed by 1.8 SED for 35 days [10]. The adaption period in the present study was 5, 7 and 9

days for the groups 0.7 SED, 1 SED and 2 SED, respectively. We therefore assume that the mild erythema in the 2 SED group was due to the shorter adaption, which was used in the present study.

The high content of vitamin D_3 in skin is expected to decline if the period between the last UVB exposure and slaughter is extended beyond the 1 day in the present study; in rats it has been shown that vitamin D_3 is released from the skin into the circulation for up to three days after UVB exposure and around 70 % of the possible vitamin D_3 is released within the first day [35].

Kolp et al. [14] investigated a single level of UVB-exposure on pigs (1.25 SED/week[1]) for 14 weeks before slaughter with the aim of investigating the effect on calcium and bone metabolism for the purpose of studying the health effects for the pigs. However, besides 25(OH)D_3 in serum, they also included analyses of vitamin D_3 in skin. They reported no effect of the UVB-exposure on the content of vitamin D_3 in skin, but they did show an increase in serum 25(OH)D_3. Compared to our study, they used less than 0.2 SED daily, which could be the reason for the different results.

Another strategy which has been investigated is the exposure of pigs to sunlight during summer in order to increase the vitamin D content in pork [13]; this study includes a control and one treatment group of 1 hour of summer sunlight per day for14 days before slaughter. An effect of sunlight was shown for content of vitamin D_3 and 25(OH)D_3 in lean pork loin, and for 25(OH)D_3 in subcutaneous fat, but no increase in the vitamin D_3 content in the subcutaneous fat was observed. Larson-Meyer et al. [13] suggest that they reached the limit of how much vitamin D_3 can be stored in the adipose tissue, however, in light of our findings this seems not to be the explanation.

During winter if above or below 35 °N and 35 °S, respectively, no cutaneous vitamin D will be produced [24]; giving pigs' access to an outdoor area will therefore not alleviate the problem with human vitamin D deficiency, which is a problem, especially in winter.

Effect of UVB exposure compared to effect of feeding trials

Maximum increase in vitamin D_3 was obtained with a daily exposure of 1 SED; compared to the control group the content in lean meat was increased 19 times. The dose of 1 SED was used in the last 19 of the 28 days the UVB trial lasted and resulted in a content of 3.7 ng/g vitamin D_3 (range: 2.3-5.3 ng/g) in lean meat; this is more than two-fold higher compared to the vitamin D_3 content of 1.2 ng/g loin from pigs fed 2000 IU/kg, which is the maximum allowed vitamin D_3 in feed in Europe [17]. The vitamin D_3 content in subcutaneous fat was found to be 120 ng/g (range 40-240

[1] 0.96 MED (minimal erythemal dose) where 1 MED is 125 J/m^2 according to their reference and thereby equal to 1.25 SED.

ng/g) that is 5-20 times higher than formerly reported values of 7.5-7.6 ng/g in subcutaneous fat from pigs fed 2000 IU/kg [16, 17]. In rind, 1 SED resulted in a vitamin D_3 content of 259 ng/g (range 170-530 ng/g) that is 55-170 times higher than the 3 ng/g reported in a feeding study at maximum feeding level [16]. The liver from pigs exposed to 1 SED had a content of 29 ng/g (range 20-45 ng/g) that is 2-5 times higher than the 8.4 ng/g in liver from pigs fed 2000 IU/kg [17]. This content was lower than in liver from mini-pigs exposed to UVB [10], but the duration of the UVB treatment differed, being 120 days for mini-pigs and 28 days in this study.

Similar to vitamin D_3 the content of 25(OH)D_3 was highest in the 1 SED group, and higher in the UVB-exposed pigs compared to the results from feeding trials using 2000 IU/kg feed [16, 17].

Unexpected increase in vitamin D_2

Unexpectedly there was found an increase in the vitamin D_2 content in the UVB treatment groups compared to the control. We suggest that it originates from either feed or straw contaminated with fungi. In another study it is shown that when grass contaminated with fungi is exposed to UVB the ergosterol in the fungi is converted to vitamin D_2 [36]. This would explain why only the groups with UVB-exposure show an increase in vitamin D_2 as the straw and the feed were exposed to the UVB when administered to the pigs and thereby functioned as a source of vitamin D_2 in the UVB exposed groups. We cannot confirm if this is the explanation, as neither the straw nor the feed were analysed for vitamin D metabolites.

Interaction between vitamin D_2 and vitamin D_3

In contrast to vitamin D_2, the control group had the highest content of 25(OH)D_2. The 25(OH)D_2 content in serum from the UVB treated groups were significantly lower than in serum from the control group. Furthermore 25(OH)D_2 was present in rind from the control group but not found in the in rind from the UVB treated groups. To our knowledge this is the first time an increase in vitamin D_3 has been reported to result in a decrease in 25(OH)D_2. Previously, only a decreasing effect of vitamin D_2 intake on 25(OH)D_3 serum concentration has been reported as summarised by Wilson et al. [37]. Lehmann et al. [38] administered vitamin D_2, D_3 or placebo to healthy humans for a period of 8 weeks. They suggested that vitamin D_2 impairs the hydroxylation of vitamin D_3 as it explained that they observed a higher decrease in serum 25(OH)D_3 in the group administered with vitamin D_2 than in the placebo group [38]. However, based on our

results we suggest that the hydroxylation of vitamin D_2 and vitamin D_3 is a classic case of competitive inhibition where the substrate present in the highest concentration inhibits the hydroxylation of the substrate with the lower concentration [39].

Effect of UVB exposure on variation in vitamin D content

The within group variation, in vitamin D content, is generally low in feeding trials [16, 17]. The variation in the control group in this study was also low, while the variation in the UVB exposure groups was higher. The relative precision (RSD) for the vitamin D_3 content measured in rind, subcutaneous fat and lean meat from the 1 SED group was 4.3, 1.7 and 1.8 times higher, respectively, compared to RSD from the control group. Similarly, sun exposure causes higher variation [13].

The effect of UVB-exposure on vitamin D content in pork possibly depends on three factors: the height of the pigs; the individual behavior, especially standing or lying; and the effectiveness of the endogenous production of vitamin D_3 in the skin. There was noticeable height difference between the pigs; the height of the individual pigs was however not measured but was estimated to be around 60 cm at arrival and 70 cm at slaughter. The higher the pig, the closer the pig was to the UVB tube and thereby the dose received would be higher giving raise to higher vitamin D production; the same reasoning applies for some pigs standing more than others. Some of the pigs had pigmentation on part of their bodies, this could in theory decrease the effectiveness of the endogenous production of vitamin D [40], though for cows no effect is observed [11].

$25(OH)D_3$ as biomarker for vitamin D content

One study has shown that there is a linear relationship between the serum $25(OH)D_3$ and the vitamin D_3 and $25(OH)D_3$ content in meat when vitamin D_3 is added to the feed [17]. In the present study serum $25(OH)D_3$ was associated to the content of vitamin D_3 and $25(OH)D_3$ in rind, fat, lean meat, liver and serum. This indicates that a model may be set up between serum $25(OH)D_3$ and the content of vitamin D_3 and $25(OH)D_3$ in pork products. Such a model could be useful in quality control of vitamin D content in pork products, as the analysis of serum $25(OH)D_3$ is easier, faster and cheaper compared to analysis of other tissues. Larson-Meyer et al. [13] also found a positive association between serum $25(OH)D_3$ concentrations and vitamin D_3 in loin (R 0.63) although not as high as ours (R 0.96). They also found a

positive association with 25(OH)D_3 in loin (R 0.83). The reason for the stronger association shown in our study might be due to the difference in analytical assays for serum 25(OH)D_3, namely RIA in Larson-Meyer et al. [13] and a chemical assay in this study. A concentration dependent difference has been shown between the two analytical principles of quantification ; the linear correlation between the two is segmented with a shift around 50 nmol/L[41, 42].

Effect of UVB exposure on weight gain

UVB exposure did not have an effect on the weight gain of the pigs. The same was found in studies where vitamin D status was followed in pigs exposed to 1 hour of sunlight for a total of 28 days, and in pigs exposed for 14 weeks [14, 15].

Strengths and limitations

The strength of our study is the characterisation of the UVB-source as well as the documentation of the UVB-exposure of the pigs during the study. Furthermore, a sensitive and specific analytical method capable of quantifying all four vitamin D metabolites was used for food and serum samples.

However, if the length of the exposure prior to slaughter had been extended beyond the 28 days it might lead to an additional increase in vitamin D as 50 days was needed to reach maximum serum vitamin D_3 concentration in mini-pigs exposed to UVB-light [10]. The UVB-tubes used had a maximum at 312 nm, however, the optimal wavelength for the production of vitamin D_3 in skin is 296 nm; at this wavelength approximately 10 times more vitamin D is produced compared to 312 nm [7]. If the spectrum of the used UVB-tubes was shifted so it had a maximum at 296 nm it would, however, only increase the erythemal effective irradiance with a factor of 3; therefore, in theory, the exposure time needed to obtain 1 SED would be 1/3, compared to at 312 nm, but the production of vitamin D_3 would increase approximately 3 times.

The variation caused by individual behavior could possibly be lowered by extending the exposure time, which could be implemented by increasing the height from lamp to pigs or using a UVB source with lower intensity.

Perspectives

It was demonstrated that it is possible to enhance the vitamin D content in pork by use of artificial UVB-exposure in indoor facilities for pigs, providing pork products with higher vitamin D content than has been shown in feeding trials. The obtained content by use of UVB-exposure is similar to the levels found in fish [43, 44]. Using UVB is a natural process for fortification with vitamin D that may be useful for marketing this pork given the public aversion to genetically modified organisms (GMO) products in Europe. Additionally, the vitamin D content is expected to be unchanged during cooking as there is no significant difference between the vitamin D content in raw and cooked pork loin with rind [45].

The potential of our results (with only 28 days of exposure before slaughter) may be expressed based on the pig with the highest content of vitamin D; the total vitamin D activity in 100 g of minced meat with 10 % fat is calculated to be 3.0 µg and in a traditional Danish liver paté (35 % liver and 15 % lard) the activity is calculated to be 4.9 µg/100g; this is enough to be labelled 'high content of vitamin D' under current European legislation (Regulation (EC) No 1924/2006). However, the maximum production of vitamin D was probably not reached as we found a linear relationship between UVB dose and vitamin D content. Therefore, the UVB-lighting method described still calls for further investigation to realise its full potential to enhance vitamin D in pork.

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Compliance with Ethical Standards

The Animal Experiments Inspectorate, part of the Danish Veterinary Food and Administration, judged that the trial did not need a governmental approval. No ethics committee was involved as the pigs lived similar to pigs at a farm except that they were exposed to UVB light similar to a daily exposure of approximately 5 to 20 minutes summer sunshine in Denmark. The project plan was assessed and approved by a veterinarian at the Department of Experimental Medicine at University of Copenhagen (Number P16-414). The animals were overseen by keepers and veterinarians during the trial.

Conflict of interest None.

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